

Fluorometric Imaging Plate Reader (FLIPR) assay 24-30 h later, the cells were loaded with 4 μ M FLUO-4/AM (Molecular Probes) and 0.04% Pluronic F-127 (Molecular Probes) in Hepes-buffered saline (HBS), 140 mM NaCl, 5 mM KCl, 2.5 mM CaCl₂, 10 mM Hepes, 10 mM glucose and 2.5 mM probenicide, pH 7.4, for 1 hour at 37°C. Thereafter, cells were 5 gently washed in HBS by an automated plate washer (Denley Cellwash, Labsystems) and transferred to the FLIPR (Molecular Devices). The FLIPR integrates an argon laser excitation source, a 96-well pipettor, and a detection system utilizing a Charged Coupled Device imaging camera. Fluorescence emissions from the 96 wells were monitored at an emission wavelength of 510 nm, after excitation with 488 nm (F488). Fluorescence data were collected 10 1 min before and 10 min after stimulation. Data were collected every 6 s before and every 1 s after agonist stimulation. 50 μ l of 3x concentrated agonists were delivered within 2 s by the integrated 96-well pipettor to the wells containing 100 μ l HBS. Agonist responses were quantified using the amplitudes of the fluorescence peaks. We averaged the 15 responses of five wells containing cells expressing the same receptor and that received the same stimulus. Calcium traces were determined in triplicate of mock-transfected cells stimulated with the same concentration of tastant. EC₅₀ values and plots of the amplitudes versus concentrations were derived from fitting the data by nonlinear regression to the function $f(x) = 100 / [1 + (EC_{50}/x)^{nH}]$, where x is the agonist concentration and nH is the Hill coefficient. The results for hTAS2R10 (Table II), hTAS2R14 (Table III), hTAS2R16 20 (Table IV), hTAS2R38 (Table V), hTAS2R43 (Table VI), hTAS2R44 (Table VII), hTAS2R45 (Table VIII), hTAS2R46 (Table IX) and hTAS2R (Table X) are shown below.